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International biobanking for lung cancer and COPD as the future resource for clinical protein research

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ABSTRACT

Characterized tissue with pathological grading and blood samples as well as other biofluids forms the basis for all biobanks as a resource in modern life science. Biobanks are accessed to measure biological components that can be used to monitor the status of health and disease in individual samples and population groups. The biomarker diagnostics area, predicting drug efficacy, stratification of patient groups, can benefit from the continuous qualitative developments, where Proteomics can make a difference in lung cancer and COPD. This in turn can provide key treasures to novel drugs for personalized medicine in the future.

1. Biobank infrastructure within healthcare

The healthcare sector throughout the world is undergoing major changes in order to meet the increasing demand of patient care. Biobanks are a major resource for scientists to access unique patient samples for medical research. Many studies within clinical proteomics utilize stored samples contained in biobanks to measure specific end points. Biomarker studies within the proteomics research field have been a major focus for many research teams. This is illustrated by a Google search where more than 3.5 million hits come up using Biomarkers Proteomics as keywords. The success and output of these initiatives has been poor, and one important reason for the limited usability of Proteomics data is most probably linked to the quality of Biobank samples from patients.

Standardizations and quality control of samples being processed for storage as well as retrieval of stored samples are important goals in order to support the development of diagnostic biomarkers [1]. Today we have not yet achieved

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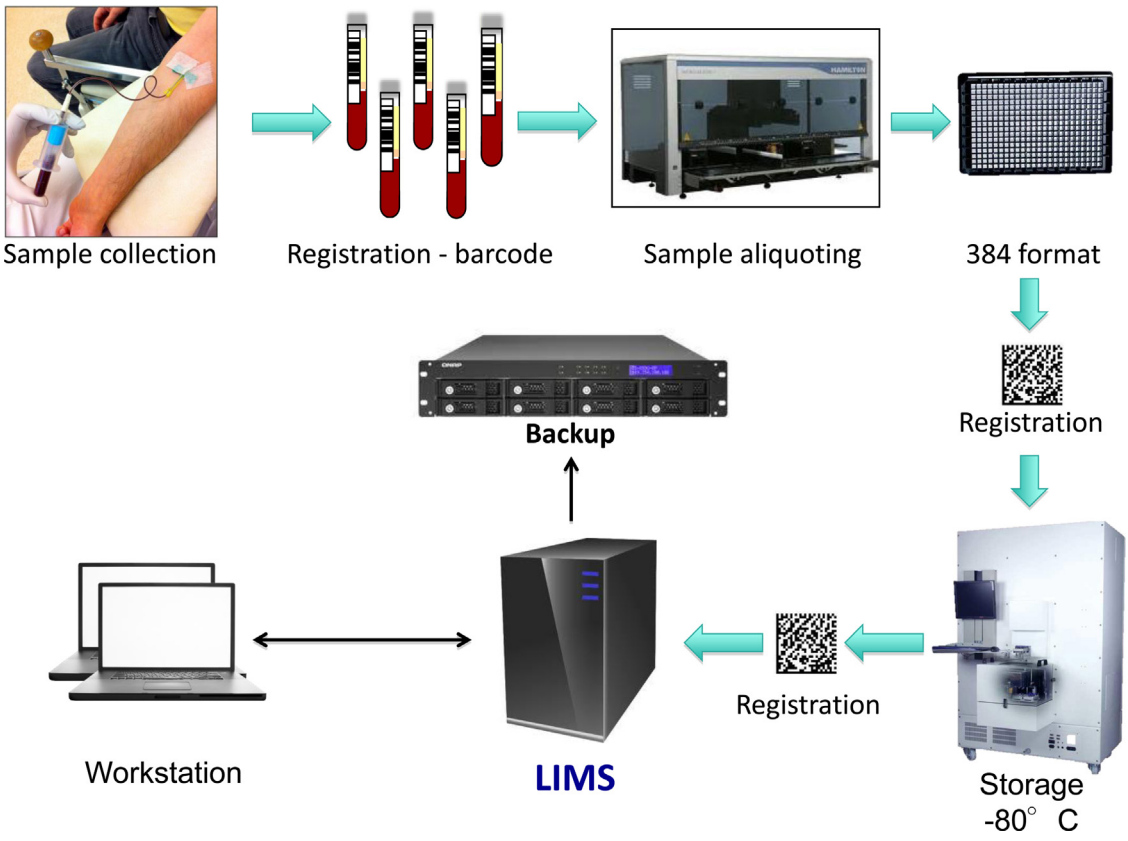

Fig. 1 – Biobank work flow where the patient sample is processed and aliquoted in high density tubes systems and stored in low temperature biobank freezer units with robotic handling and processing at −80 °C.

consensus on how to collect, manage, and build biobank archives in order to reach goals where these efforts are translated into value for the patient. This is currently a challenge where lack of available high quality sample collections with a wide range of disease cohorts has become a rate-limiting step for drug development, medical research and novel diagnostics. In order to provide best support to patients, it is clear that improvements and developments within healthcare will be closely linked to the introduction of new technology platforms. Novel technologies as improved tools and concepts will be associated with the ability to preserve the sample integrity of bio-samples that are stored for periods that will reach one or several decades as illustrated in Fig. 1. In addition, the availability of samples that are available as a global resource within a documented Biobank repository will be key, in order to perform comparative studies where we make use of clinical data that are associated to patients, such as metadata of measurements and clinical phenotyping. These progressive developments were recently presented in several international initiatives in order to disseminate best practice examples for biobanking [2,3].

Today in Europe, the Biobanking and Biomolecular Resources Research Infrastructure (BBMRI) currently has a 54-member consortium by means of 225 associate organizations from over 30 countries. International utilization of bio-samples across country borders serves as integrated resources, using bio-repository patient material to develop healthcare needs of tomorrow. The outstanding demand in most countries in hospitals is putting a high demand on modern healthcare, where the fast progress in clinical research has created a large demand for blood, tissue and other clinical samples used in genomics and proteomics studies [4]. Today we invest hundreds of millions of dollars internationally on the collection, storage and distribution of samples. Biorepository archives in Europe, as well as other continents, rest on best practice experience, as well as quality assurance based tests and assays, without any agreed and approved guidelines. We have been able to resolve that by introducing basic standardization requirements and protocols, as an initial quality assurance guideline [5,6]. Recently, a large-scale 384-format biobank sample tube system was introduced that will allow us to make global Proteomics multi-center studies [7]. Since the patient blood sample volumes are low, 50–70 μL, multiple aliquots are being collected from each blood fractions, e.g., plasma, leucocyte fraction, serum and whole blood [8].

In clinical practice today we have more than 100 protein targets that are utilized for clinical assay screening, from where a combination of biomarker read-outs will be used as the basis for medical diagnosis of patients [9].

In order to regulate protein expression data output and build on clinical disease value for patients, there are biobank

laws attached, to regulate these activities in each respective country. The biobank documents are very extensive and regulates the way that clinical samples and the resulting outcomes can be use and the utility of patient samples that has been collected in large well-organized biobank archives. Lately, the specific requirements that are needed for Biobank developments with respect to patient sampling workflow, has been taken into consideration and dedicated protocols to optimize the stability of proteins has been taken into practice (http://lifescience.skane.org/content/new-development-offers-unique-and-increased-opportunity).

Usually, informed consents from patients are the basis for any bio-sample use in clinical science, where the patient has been informed on the objectives and future healthcare use for fellow patients. Consequently, documentation regulates the availability of the sample usage. In most cases, this is declared in a clinical study protocol, or other types of study documentation. The practical parts are handled in a manner that promotes integrity and that is aligned with legal considerations.

International guidelines that are regulated by biobank laws and approved by ethical boards are already today common practice that the research community as well as the authorities like FDA and EMEA live by. What is in progress in order to capitalize on the Biobank investments are the documentation and quality aspects that will drive the usability of all these stored samples that is a resource treasure well stored.

2. Lung cancer and personalized drug treatment

2.1. *Molecular phenotyping: the association of drugs with tumor type*

A paradigm shift was witnessed in the management of lung cancer in the past decade due to two factors. Pathological diversity of lung cancer was translated to histology-associated chemotherapeutic protocols recognizing the basic entities as squamous cell cancer, adenocarcinoma and small cell lung cancer (Fig. 2A). Parallel to these developments, a molecular sub-classification of lung cancer was started to be established which is now detailed enough in adenocarcinoma but starting to be useful in other histological variants as well. Molecular classification of lung adenocarcinoma revealed that the major driver mutation is K-RAS [10] followed by EGFR, B-RAF and ALK-fusions [11–13] where several efficient therapeutics are already available (Fig. 2B). As a consequence lung cancer is now an example of personalized medicine where target therapy improved progression-free as well as overall survival of lung cancer patients, now frequently allowing second or third line of treatments. However, the basis of this development was the close collaboration of pathology, pulmonology and molecular biology. Unfortunately, lung cancer is the only major human cancer where cytological diagnosis is still

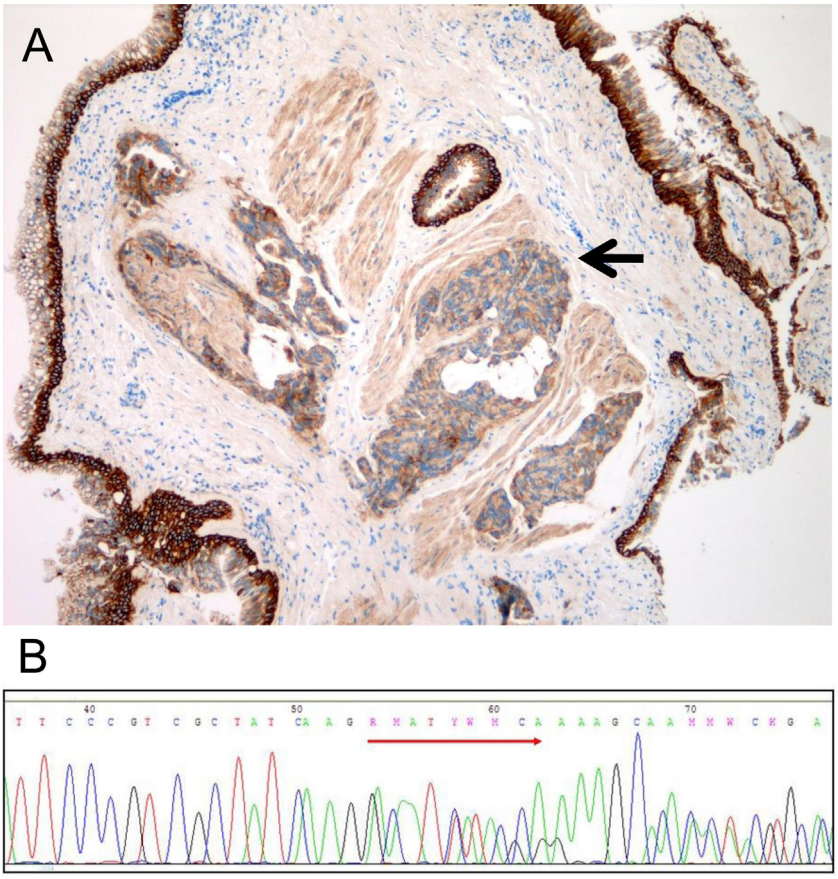

Fig. 2 – Transbronchial biopsy specimen. (A) Normal epithelium exhibits strong EGFR protein expression while adenocarcinoma exibits a EGFR-negative status (arrow). (B) Sanger sequencing of EGFR exon 19 in lung adenocarcinoma TPL 52/11. Note the activating mutation at c2236_2250*del*15 (cosmic ID:6225).

significantly frequent and where the primary tumor is inoperable in the majority of cases. Accordingly molecular diagnostics and biobanking of lung cancer provide the major challenges due to insufficient amount of primary tumor tissue. Furthermore, small biopsy samples or cytological smears are not representing the pathological and molecular heterogeneity of the tumor. These issues can be overcome by heavily relaying on metastatic samples or body fluids such as pleural effusions or blood serum. While lung cancer biobanks are already serving the successful therapy of patients, the rapid development of molecular pathology and target therapy of lung cancer is also due to these resources further justifying the concept.

The first personalized medicine for lung cancer patients was the tyrosine kinase inhibitor Gefitinib (IRESSA) [14], introduced in Japan, where follow-up protein biomarker studies were initiated and reported on [15]. Protein markers as targets for predicating optimal patient efficacy and safety has been the center of research for a number of years where the drug development goes from traditional chemotherapy, radiation treatment and to the new generation of targeted treatments. The present status and best practice of clinical proteomic analysis for early detection and determination of therapeutic strategy in lung cancer was outlined and introduced, as an example of protein expression strategies [16–18]. A recent paper by Kato et al., illustrates the status of proteomics studies and initiatives over more than a decade with a focus on the clinicians view and worked out experiences of proteomic studies in the Light of Lung Cancer [12].

3. COPD grading and disease pathophysiology

Chronic obstructive pulmonary disease (COPD), a heterogeneous and progressive pulmonary disorder characterized by airway obstruction, systemic manifestations and increasing frequency and severity of exacerbations, affects more than 0.2 billion people worldwide and is expected to rise to the third leading cause of death by 2030 [19]. Although COPD is mainly caused by smoke inhalation, it has become obvious that other noxious agents such as burning biomass fuels in poorly ventilated homes can also play a role in its development [20]. Nevertheless, the mechanistic concepts that have been implicated in the pathogenesis of the disease include: (i) chronic airway inflammation in response to inhaled noxious particles, (ii) host factors including genetic susceptibility, epigenetic changes, and oxidative stress, (iii) an imbalance between proteases versus antiproteases and oxidants versus antioxidants leading to a net increase in proteolytic activity, oxidative stress and apoptosis of structural cells. These mechanisms, however, do not operate separately; in fact, they are interlinked, participating concurrently in COPD progression [21]. Recent protein expression studies have provided evidence of proteins that have been differentially regulated, with clear implications to a disease link [21].

Similarly to lung cancer, a major challenge in COPD drug development is to identify molecular and imaging-based biomarkers that could be used to define surrogate outcomes in clinical trials and, moreover, to identify those patients who are likely to benefit from emerging drugs [22]. Biomarker studies in complex disorders like COPD, however, have to overcome the challenge of disease heterogeneity. In addition, although there is an obvious need for higher case numbers in COPD phenotyping studies to overcome this obstacle, this need is further challenged by the small number of adequately preserved pulmonary tissue samples available. Thus, because only high-quality biological samples can provide the data needed for biomarker research, biobanking has also a key role in developing more effective anti-COPD strategies. In the European Union funded EvA (emphysema versus airway disease) study, we are investigating more than 800 biobanked samples from both cases and controls [23]. Our consortium aims to identify novel markers for COPD and its main phenotypes (i.e., emphysema and airway disease), by analyzing bronchial and alveolar gene expression patterns in COPD patients and matched controls. To this aim, patients go through pulmonary function analysis and chest CT (used to define the phenotypes based on lung density and airway wall thickness). Then bronchial brush and lavage samples taken by bronchoscopy along with blood samples are being subjected to genome-wide expression and association analysis and biomarkers linked to the phenotypes are identified.

4. Conclusions

Looking into the pipeline of forthcoming drug products, personalized medicines, as well as antibody-based biopharmaceuticals are anticipated to grow significantly. The most important part of any drug development is the access to high quality clinical samples. Both specific tissue compartments, characterized by pathology, blood samples and other biofluid samples and specimens are the basis for all biobanks as a resource. By continuous improved standardization and qualitative developments will provide key treasures to novel drugs in the future. The biomarker diagnostics area, predicting drug efficacy, stratification of patient groups, and aiding in safety assessment, will also grow as a result of these developments. All of these changes for the better in modern healthcare will be driven from successful implementation of translational science, progressing both in the pharmaceutical and biotechnology industry, as well as within academic research.

Recently, a report came out that proposes Proteomics strategies in order to improve international research biobank sample usage of clinical specimens [24].

One should also bear in mind that the value of the samples is dependent upon developing workflows that enable their use. The Human Chromosome Initiative as well as other global research activities is looking for best practice and applicability that can bring value and bridge the data sets of information held with each clinical sample. One of the major objectives is to build data collections that can be used to create future paradigms of healthcare and treatment modalities [25,26].

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